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ing perithecium; osteole poreiform, innate. Epithecium pale, whitish. Hymenium black. Spores ellipsoid, elongated, hyaline, bilocular $\frac{1.4 \times 1.6}{4 \times 6}$ mic.

On trunks with smooth barks, San Luis Potosi, Mexico. Mr. C. G. Pringle. Not unlike *P. majuscula* Nyl. of the Hawaiian Islands in general appearance.

Notes on the Chromatophores of *Astrophyllum sylvaticum* Lindb. (*Mnium cuspidatum* Hedw.), and of some other Plants.

BY ALFRED C. STOKES.

Among the commonest mosses in my locality is the one usually referred to as *Mnium cuspidatum* Hedw., my specimens having been identified for me by Dr. G. N. Best, of Rosemont, N. J. The plant is always so attractive in appearance at almost any season, that for several years I have been in the habit of collecting it in the autumn, and of keeping it all winter under an inverted glass on my table, where in the diffused light of a warm room it will grow and flourish, and, as I have learned, supply the microscopist with several important objects for investigation, asking in return only that it shall be abundantly furnished with the moisture which it must have or die. I know that it has long been a favorite object of study with microscopical botanists, but I have not been able to learn that the observations which I have had the pleasure of making over it have been anticipated.

But the leaf-cells of *Astrophyllum sylvaticum* are so transparent, especially in the adult and the young conditions, that microscopical examination may be made of them in their living state. In these young and in the mature, but not old cells, the chromatophores (chloroplasts or chlorophyll-bodies) are so few and so conveniently scattered over the upper and the lower cell-surfaces that they may be studied with some ease and the protoplasmic cell-contents examined with as great comfort while the whole leaf is living in a 4 per cent. solution of sugar, in which its structure is apparently unaltered, and where for a time its functions are not vitiated. It is especially to the structure of the chloroplasts in these and in some other leaves that this paper is devoted.

There has been considerable discussion as well as considerable divergence of opinion in regard to the structure of the chlorophyll-grains. What I have to relate refers solely to what I have seen, or think that I have seen, in the chromatophores of the plants to be mentioned.

Any countryman is entitled to an opinion on all subjects, provided that the tools which he uses in his investigations are as good as can be had, that he uses them intelligently and that he describes his observations as he thinks he has seen the objects, holding his imagination in check, and always bearing in mind that he is human and therefore "prone to err." The tools in this special case are the $\frac{1}{4}$ and the $\frac{1}{8}$ inch objectives, both by Zeiss, and both apochromatic; Spencer's homogeneous-immersion $\frac{1}{10}$, N. A. 1.35; Reichert's semi-apochromatic, oil-immersion $\frac{1}{12}$, N. A. 1.40; Gundlach's homogeneous-immersion $\frac{1}{20}$, N. A. 1.22; an achromatic condenser; Powell and Lealand's 2-inch, and Zeiss' 8 and 27 eye-pieces, all compensating. The conditions are that the leaf shall be studied in the 4 per cent. sugar-solution with no preparation, except in some cases, the free-hand sectioning of the object and its immediate examination.

In reference to the structure of chloroplasts, F. Schwartz says* that they are formed of fibrillæ, which are arranged in a network, yet lie side by side attached to one another by what he calls metaxin. Such structure is not visible in the chromatophores of *Astrophyllum sylvaticum* nor in those of any other plant which I have examined; and likewise A. Meyer says† that he has failed to see the fibrillæ and does not hesitate to add that Schwartz's observations are incorrect. Schimper, in an extended treatise on the subject says‡ that the simplest chromatophores consist of a colorless protoplasmic substance without any visible internal structure or contents, and that this is sometimes the case during the whole of their existence, as with most leucoplasts.

Structure is so plainly visible on the external surfaces of the chloroplast of *Astrophyllum sylvaticum* that I confess to consider-

* Cohn's Beitr. z. Biol. Pflanzen, 5: 1-224.

† Bot. Ztg. 46: 636.

‡ Pringsheim's Jahrb. f. wiss. Bot. 16: 1-247. Cf. Journ. Roy. Micros. Soc. (II.) 6: 640.

able regret when I failed in my efforts to section the chlorophyll-grains, or rather to assure myself that they had been sectioned after the effort to cut them had been made. Yet optical sections obtained with the best objectives of wide angle have always so plainly showed the structure from one surface to the other that I must believe that what appears so conspicuously on the outside is likewise continued throughout the entire chromatophore. I have not yet seen a chloroplast from this special moss, nor from any other plant in which they are externally structured, in which I have failed to observe the markings until the chromatophore became disintegrated; in sections where the chlorophyll-grains were presumably cut, homogeneous, internal substance has been equally invisible.

That the pitted or spongy aspect so apparent on the surface of the chloroplasts of *Astrophyllum* is continued within the internal substance is demonstrable only by means of the optical sections referred to, since the chlorophyll-grains are so loosely attached to the cell-wall, never coming into actual contact with it, but being imbedded in the threads of protoplasm which radiate through the cell, that they are forced out of the section by the pressure of the knife, and are either not positively cut or are lost. That the entire chromatophore is a chlorophyll-bearing, spongy body cannot, I think, be doubted, after a careful study with good objectives.

The surface-structure referred to, covers the entire chloroplast with an irregularly disposed series of depressions of unequal size and shape. These depressions are surrounded or produced by narrow elevations of the chromatophore-substance, the green coloring matter being as conspicuous in each of the delicate, ridge-like elevations as it is in the general body-substance of the chloroplast itself. When the chlorophyll-grain is examined in optical section, at irregular intervals around the entire margin of the body there come into view the edges of the ridge-like elevations which form the boundaries of the pits, and these appear as exceedingly delicate, green bars projecting from the body. The general appearance, when the chlorophyll-body is examined in this way, is remotely like that of the endocarp of certain stone-fruits, the surface of a peach-pit, for instance, the depressions on the surface of the peach-stone of course being even less regularly disposed and

more shallow than are those of the chloroplast. If an optical section of the peach endocarp could be obtained, the ridges which surround the depressions would, to a certain extent, produce the delicate, bar-like projections as on the margins of the chloroplasts.

My attempts to make drawings of these surface-appearances have failed, although I have had the assistance of beautiful photomicrographs prepared for me by Dr. H. G. Piffard, of New York, a most accomplished photo-micrographer. The structure is so delicate and so minute that even the photo-micrographs reproduced by the half-tone process would not satisfactorily show it. To see it with the microscope certain conditions are required.

These conditions must be complied with, or the depressions, as I believe them to be, will appear as whitish elevations comparable to the "pearls" on the diatoms when these plants are resolved in the common, that is, the incorrect way. These chromatophore-pearls, like the diatom-pearls in certain cases, become black depressions when the objective is properly focussed. If the apparent elevations on certain parts of certain diatoms are not elevations, then the apparent pearls as seen with a certain focus on the chromatophores are not elevations. In both cases they must be depressions or apertures; otherwise the entire fabric of the secondary structure of the diatoms falls to the ground. If the surface of the chromatophores of *Astrophyllum sylvaticum* is not deeply pitted, and if the entire structure, as seen in optical sections, is not similar to the trabecular formation of the ordinary sponge-skeleton, then those of us that have taken pleasure in the study of the secondary structure of the diatoms have had all our work for nothing, and have been pursuing a phantom. But the existence of this secondary diatom-structure can be demonstrated beyond a peradventure.

The lowering of the objective to obtain this "black-dot" resolution gradually brings into view the blackness of the depressions, with the well-defined outlines of the narrow elevations which surround them. The surface of the chromatophore then has a spongy appearance, the black spaces between the irregular network seeming to enter the substance of the chloroplast and there to produce, not a structureless, homogeneous body, but a body of green meshes. Those that have studied the secondary structure of cer-

tain diatoms are familiar with the fact that when the whitish pearls have become black dots by the proper focussing, the edges of the black apertures are not always sharply cut; in fact there is no actual, well-defined edge to the opening, but it is approached by a concave slope, a smooth depression which surrounds the black aperture and leads to it. A homely comparison may be made by thrusting the finger-end into stiff dough, when the resulting depression will show an evenly rounded approach similar to that so readily observable in certain diatoms. A similar condition exists with the depressions on the chromatophores of this moss.

This spongy aspect is not an optical illusion produced by minute granules or other objects within the protoplasm of the cell. The pits and the laterally projecting bars are as conspicuous when the chromatophore has been forcibly extruded and is floating freely in the mounting medium as they are while within the living cell. But the pits are exceedingly shallow; and the narrow ridges are correspondingly low, for I have not been able to measure them with the ordinary micrometer, as the lines made by the diamond are so broad in comparison that they obliterate the bar-like points of the optical section.

That the chromatophores of all plants are trabecular in structure is not yet to be written. In many of our common plants it cannot be demonstrated. In those of *Astrophyllum sylvaticum* there can be no doubt of the trabeculæ.

There has also been some discussion and some disparity of opinion as to the existence of a membrane surrounding the chlorophyll-grain. That all chromatophores are surrounded by a membrane is another assertion which must be put to the test by examining the chlorophyll-grains of all species of plants. Yet Tschirch says* that after experiments on the living cells of *Elodea* (*Anacharis*) and of *Nitella* he is convinced that all chlorophyll-bodies and aleurone-grains are surrounded by an envelope. Repeating this statement in another place† he adds that the coloring matter of two different grains never comes into contact. This is a part of the morphology of the chlorophyll-grains of *Astrophyllum sylvaticum*, and of those in some other plants. Yet in the moss

* Ber. Deutsch. Bot. Gesell. 1: (1883).

† Ib. 2: 265.

the membrane is so exceedingly delicate that it is scarcely possible actually to see it in either the young or in the old chloroplasts. But in those which are undergoing the act of bipartition, this surrounding membrane may at times be examined with some ease, although those fortunate times are not common. In such chromatophores I have seen the membrane, but it becomes especially apparent in those which have been treated with alcohol to bleach the chlorophyll, followed by staining with eosin; fig. 2 representing an optical section of a chlorophyll-grain after such treatment. The two grains are completely divided with the exception of the enveloping membrane which still binds them together. The alcohol has contracted the chromatophore, thus leaving the delicate membrane visible not only about and between the dividing planes, but around the margins of one of the chloroplasts, where it is, as usual, prevented from coming into contact with the body of the grain by the projecting ridges of the surface-structure. On the smaller chromatophore at the left-hand side the membrane is not visible, but in the space separating the two grains it passes as a distinct but exceedingly delicate line, the space being bounded above, as shown in the figure, by a membrane conspicuously depressed in the middle, the lower boundary being as plainly developed.

In fig. 4 is shown a greatly enlarged drawing of the inner ends of the same chloroplasts with the central membrane. The distance from one chlorophyll-grain to the other was about $\frac{1}{8000}$ inch. I have no means of measuring the thickness of the central membrane which separates the chloroplasts, but that membrane is double. This is an exceedingly "fine point" microscopically, and it is here that the "black-dot" resolution again makes itself useful. If the two membranes had been undivided or in actual contact throughout their whole length, the double structure would of course have been invisible. That they were not thus in actual contact the "black-dot" resolution demonstrates by exhibiting the minute black spaces near the upper and the lower extremities, as the chromatophores lie horizontally across the field, the attempt to show this on the greatly enlarged scale in fig. 4 being hardly more than a diagram. In fig. 2 the black, irregular line around the edge of one chromatophore represents the space between the

body of the chlorophyll-grain and the exceedingly delicate membrane, the white projections being the optical sections of the elevated ridges which give the surface its pitted aspect.

In fig. 3 is shown an isolated chromatophore in optical section after treatment with alcohol, by which the grain has shrunk, while a few of the ridges have become rather more prominent, the outline here representing the surrounding membrane, the space between, in the microscopical specimen, being black.

If such a microscopically broad space of darkness is an optical illusion, I am at a loss to account for it on any optical principle with which I am familiar. If it is an optical illusion, why the upper margin of the black band should slope from the long projection near the upper right-hand border of the chloroplast to the minutely projecting point at the very apex of that corner, and form concavities between succeeding elevations, becomes even more inexplicable. The enveloping membrane exists.

In addition to the membrane there is a coating of the cell-protoplasm in which each chlorophyll-grain is imbedded. Yet each chloroplast is not entirely isolated from all the others, but is intimately connected by the peculiar arrangement of the protoplasm, which does not entirely fill the cell as it frequently does in similar bodies, but forms a kind of protoplasmic cobweb which loosely fills each cell with its delicate threads. It is these protoplasmic threads which connect together the chromatophores, and it appears to be by their contractions that the position of the green bodies is changed under the influence of the light. A somewhat similar arrangement of the protoplasm has been observed in *Selaginella* by Haberlandt;* a part of one of his drawings is shown in fig. 1.

In *Astrophyllum* these protoplasmic threads are colorless, variously branched and in continuous movement. Every chromatophore in the cell is connected by their means with every other, and the threads themselves radiate from the nucleus.

In fig. 5 are shown several leaf-cells with the protoplasmic threads extending across and around, some being in the central parts of the cell and therefore having no chromatophores in their course. These delicate filaments are continually quivering,

* Flora, 71: 221.

lengthening and shortening, extending themselves in long curves or loops, or contracting into short lines or into little rings. When the leaf-cells are active, the protoplasmic meshes are so numerous and so conspicuous that the entire cell seems to be crowded with the network, within the threads of which float the chromatophores like minute green sponges in a colorless, ever quivering jelly.

Fig. 7 shows the nucleus and its connection with the protoplasmic filaments. Fig. 8 is a transverse vertical section of a leaf to show the chromatophores arranged, as they usually are, near the upper and the lower cell-walls, with the loose network of protoplasmic threads and in the central cell, the nucleus temporarily adhering to the cell-wall. The leaf varies in thickness from $\frac{1}{1250}$ inch at the margin to $\frac{1}{700}$ near the midrib.

Even in the oldest cells the chromatophores are seldom in contact even by their enveloping membranes, and if in a healthy condition there is always a thin layer of protoplasm enclosing and separating each from all the others, and separating each from its apparent contact with the upper and lower cell-walls. When two chromatophores are in apparent contact, careful scrutiny with a high magnifying power will exhibit the black spaces between the peripheral trabeculæ, or the bright, continuous line which is present between two chloroplasts in which the act of bipartition is nearly completed.

Ageratum conyzoides L. cultivated variety *Mexicanum*. In the mesophyll-cells of this plant the chromatophores have a structure similar to that of the chlorophyll-grains of *Astrophyllum sylvaticum*, the larger having more conspicuous trabeculæ, and the surrounding membrane, which exists here also is raised to a shorter distance above the body of the chromatophore on account of the lower elevation of the surface-ridges, or of the network of elevated lines between which the depressions or apertures, as I believe them to be, are placed.

Fig. 6 is a diagram of two chloroplasts from this plant in an advanced stage of bipartition, with the central apertures somewhat exaggerated, as also are the surface depressions represented by the black dots. Attention is asked to the demonstration of the surrounding membrane where it extends from one chromatophore to

the other, and is apparently continuous with the external edge of the dark space which defines and separates the optical sections of the projecting ridges.

Mesembryanthemum crystallinum. (Cultivated.) The chromatophores here seem to have a spongy structure similar to that of the chlorophyll-grains of the two plants previously mentioned, but they are so sensitive to adverse influences that, although the trabecular appearance is conspicuous and distinct, it may possibly be produced by another cause. The 4 per cent. solution of sugar sometimes distorts the chloroplasts, and disintegrates them speedily; water often acts even more quickly, and when they are forced out of the cell and examined in the cell-sap, the changes are almost as rapid and disastrous to their integrity of form and of structure. Yet, although necessarily examined in such unpropitious surroundings, they seem to possess the pitted surface and the delicate enveloping membrane.

Heliotropium Peruvianum L. (Cultivated.) The chromatophores are here prominently trabecular, or spongy in structure. An enveloping membrane can be demonstrated at the periphery of an optical section.

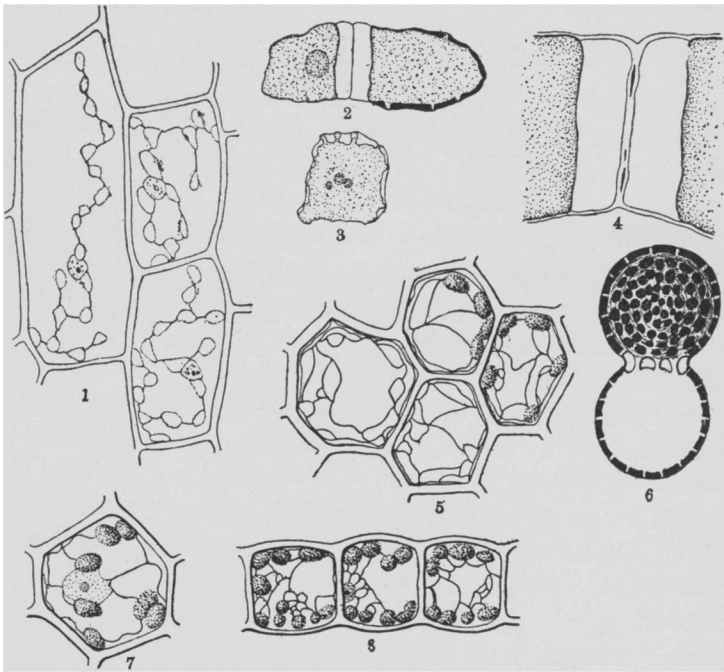
Begonia sempervirens. (Cultivated.) Here the chlorophyll-grains are large and the reticulated structure is prominently developed. At this writing I have not seen the chloroplasts of any plant so conspicuously pitted, and, as I am convinced, so plainly cancellated in structure throughout the entire chromatophore. It is not a difficult task practically to cut successive optical sections from the upper surface of the chloroplast to the lower, and in these circumstances, with these special objects, the cancellous structure extends through the whole body and is not mere surface pitting. These chlorophyll-grains offer one of the most convincing demonstrations of this interesting point that I have thus far observed.

Here, as elsewhere, the green coloring-matter is not contained in the meshes of the structure, the meshes being the spaces formed and bounded by the threads or bands which together form the chromatophore, but is within those filaments themselves. The meshes, by the correct focus of the objective, are jet black.

A membrane also seems to surround the chlorophyll-grains of this variety of *Begonia*, but is not commonly visible, being demonstrable only with certain of the larger chromatophores.

In the young leaves of other varieties of the genus a similar structure is readily visible, but in the mature and in the old the appearance becomes obsolete. The change seems to begin early in the life of the chlorophyll-grain and to advance rapidly, the chromatophore soon becoming filled with starch. That the action of the light which augments the formation of the starch has any concomitant influence on the trabecular structure of the chromatophore itself I do not know.

This structure of the chlorophyll-grain is probably not uncommon.



Explanation of Figures.

FIG. 1. *Selaginella Krusiana*; chromatophores connected together by short, delicate threads of protoplasm. (After Haberlandt.)

FIG. 2. Chromatophore of *Astrophyllum sylvaticum* in optical section, showing the surrounding membrane between two dividing grains, with optic sections of the elevated ridges and of the black space between the body of the chromatophore and the membrane.

FIG. 3. Optical section of a chromatophore of *Astrophyllum* after treatment with alcohol and with eosin staining; showing the projecting elevations or ridges and the external membrane, the space between the body and the membrane being left white.

FIG. 4. Greatly enlarged view of the central portion of Fig. 2, showing dark spaces within the membrane between the dividing chromatophores; this part of the membrane is becoming double.

FIG. 5. Protoplasmic threads in the leaf-cells of *Astrophyllum sylvaticum*.

FIG. 6. Dividing chromatophores of *Ageratum conyzoides*, showing the pitted surface, the laterally projecting ridges (in optical section) and the central spaces between the dividing chromatophores. Diagram.

FIG. 7. Leaf-cell of *Astrophyllum*, showing nucleus, chromatophores and protoplasmic threads.

FIG. 8. Transverse vertical section of leaf-cells of *Astrophyllum*, showing chromatophores, nucleus and protoplasmic threads.

Proceedings of the Botanical Club, A. A. A. S., Brooklyn Meeting, August 15-22, 1894.

The meetings were held in the Packer Institute.

FRIDAY MORNING, AUGUST 17TH.

The meeting was called to order by Professor N. L. Britton, and in the absence of the President, Prof. D. H. Campbell, Prof. Geo. F. Atkinson was elected to the office of President.

Prof. F. C. Newcombe was elected to the Secretary's office in the absence of Mr. W. T. Swingle.

The report of the Secretary was read, showing for the year ending Aug. 14, 1894, receipts to the amount of \$8, and an equal amount of disbursements. The report was accepted. Voluntary contributions to the amount of \$7.32 were made by those present.

Prof. C. E. Bessey then gave some notes on the germination of macrospores of *Marsilia vestita*. These were germinated in water, forming larger prothallia with rhizoids than are usually present in this genus.

Dr. Erwin F. Smith recommended the preliminary treatment of vegetable sections with tannin solution, in order by subsequent staining to differentiate micro-organisms present in the tissues.

Prof. F. C. Newcombe also spoke favorably of tannin solution as aiding in the differentiation of protoplasmic structure by subsequent staining.

Dr. W. J. Beal showed how measurements of parts may be helpful in the identification of grasses.